Euphytica (2006) 148: 319–330
DOI: 10.1007/s10681-005-9040-0

Molecular genetic evaluation of sorghum germplasm differing in response to fungal diseases: Rust (*Puccinia purpurea*) and anthracnose (*Collectotrichum graminicola*)

Ming Li Wang[1,*], Rob Dean[2], John Erpelding[3] & Gary Pederson[1]
[1]*USDA-ARS;* [2]*University of Georgia, Plant Genetic Resources Conservation Unit, 1109 Experiment Street, Griffin, GA 30223-1797, USA;* [3]*USDA-ARS, Tropical Agriculture Research Station, 2200 Pedro Albizu Campos Avenue, Mayaguez, PR 00680-5470, USA (*author for correspondence: e-mail: mwang@ars-grin.gov)*

Received 13 October 2004; accepted 18 October 2005

Key words: fungal diseases, molecular genetic evaluation, sorghum germplasm, SSR

Summary

To evaluate genetic diversity in relation to rust and anthracnose disease response, ninety-six accessions were randomly selected from the core collection database of the Germplasm Research Information Network (GRIN) and characterized by a set of 40 SSR markers. The mean value of polymorphism information content (PIC) was 0.8228. Two dendrograms were generated from the molecular genetic data and field morphological data, respectively. The genetic dendrogram demonstrates that the accessions can be classified into three main clades and nine subgroups. The branched subgroups correlated very well with the locations where the accessions were collected. Geographical origin of accessions had significant influences on genetic similarity of sorghum germplasm. Out of 96 accessions, only eight accessions were highly resistant to both rust and anthracnose. All the accessions from South Africa and Mali were highly resistant to anthracnose. The information from genetic classification would be useful for choosing parents to make crosses in sorghum breeding programs and classifying sorghum accessions in germplasm management.

Introduction

Sorghum bicolor (L.) Moench is the third and fifth most important cereal crop grown in the United States and in the world, respectively and is used as an important source of food in many regions in Africa and Asia. Sorghum is well adapted to environmental extremes, such as drought; however, the value and productivity of sorghum crop can be reduced greatly by sorghum diseases (Frederiksen, 2000). Sorghum rust (*Puccinia purpurea*) occurs widely in almost all of the sorghum growing areas of the world. The rust itself has a minimal effect on sorghum yield, however, it can predispose plants to other major diseases (such as *Fusarium* stalk rots, charcoal rot and grain moulds). From F_2 segregation analysis, it was projected that resistance to sorghum rust was controlled by three major genes (Rana et al., 1976). Several chromosomal regions (or QTLs) for rust resistance have been identified by interval mapping using recombinant inbred lines (Tao et al., 1998). Anthracnose (*Collectotrichum graminicola*) has been the most common and destructive disease affecting sorghum, especially when sorghum is grown in hot and humid areas. It not only can cause death of seedlings, leaves, stems, peduncles and grains but also causes stalk rot and lodging. Anthracnose can cause up to 50% of yield losses in susceptible cultivars (Thomas et al., 1996).

Although random amplified polymorphic DNA (RAPD) markers have been used to screen individual resistant and susceptible genotypes (Panday et al., 2002), limited information is available on genetic diversity and inheritance of resistance for anthracnose (Casela et al., 1993; Boora et al., 1998). Microsatellites,

Table 1. Selected sorghum accessions arranged in a 96-well format

R\C	1	2	3	4	5	6	7	8	9	10	11	12
A	563053	563164	562253	586075	585312	585855	BTX623*	291237	253632	267519	453691	284971
B	563151	562824	562990	266952	563406	586447	ATX3042	291236	248334	267515	513996	267643
C	562982	562970	562703	562252	563210	685739	ATX623	253640	267410	267447	455013	287597
D	563043	563131	562834	563032	563254	585650	585787	248336	266955	291382	514120	287578
E	562960	562065	563399	564163	585644	585795	586482	229861	267106	297129	514015	285194
F	562872	267322	563463	585813	563549	236286	195039	197542	267461	453053	267633	266063
G	563431	560388	562724	287595	585341	161586	170794	221728	267442	297094	282829	514140
H	563249	562818	514367	563269	562739	168501	291377	229850	267593	453831	287598	521702

R\C: rows from A to H, columns from 1 to 12; the number in each cell represents either plant introduction (PI) number or a breeding line.
*BTX623 = 564163 as internal controls.

simple sequence repeats (SSRs) are highly polymorphic, abundant and fairly evenly distributed throughout the euchromatic part of genomes. These properties have made microsatellites one of the most popular genetic markers for mapping and population genetics (Schlötterer, 2004). Molecular genetic evaluation of sorghum germplasm differing in response to these two fungal diseases using SSR markers would benefit sorghum improvement programs. The objectives of this study were: (1) to genetically characterize and classify the selected accessions; (2) to observe whether their responses to rust and anthracnose relate to genetic classification; and (3) to choose potential mapping parents according to genetic classification for generating a segregation population to localize chromosomal regions responsible for rust and anthracnose resistance.

Materials and methods

Plant germplasm accession and DNA isolation

Among 96 sorghum accessions, 92 from the core collection were randomly selected by searching the Germplasm Research Information Network (GRIN) web site (http://www.ars-grin.gov/npgs/searchgrin.html) and 4 were obtained from Dr. John Erpelding (Table 1). The first 92 accessions had complete data in GRIN for the following six traits: rust and anthracnose response, endosperm texture, flowering time, grain mold and race (Table 2). The descriptors for rust and anthracnose response (from resistant to susceptible) were scored 1 to 5, for endosperm texture (from corneous to starchy) 1 to 5, for flowering time (from very early to very late) 1 to 6, for grain mold response (from very good to very poor) 1 to 5 and for race and/or working group classified into 14 categories (according to the classification by GRIN), respectively. They were either cultivars or landraces. The last four accessions were two male sterile lines (ATX3042 and ATX623), one maintainer line (BTX623) and one US cultivar (PI 564163). ATX623 and BTX623 were genetically identical except for the source of cytoplasm. The accession PI 564163 was genetically the same to BTX623, only maintained separately and used as an internal control in this study.

Fifty seeds (collected from the same plot and morphologically similar to each other) for each accession were obtained from USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, GA, or from Tropical Crop Agriculture Research Station, Mayaguez, PR. The seeds were wrapped in wet paper towels and transferred into the growth chamber kept at 70% relative humidity, germinated and grown for five to seven days (for each day, 30 °C for 8 h light and 4 h dark, then 20 °C for 12 h dark). Fresh tissue (including young leaves and stems) was collected from the plants (50 plants for most of the accessions), bulked and placed into plastic bags, quickly frozen in liquid nitrogen, then transferred to and stored in a −80°C freezer for DNA isolation. Frozen tissue was ground to a fine powder in liquid nitrogen and DNA was extracted according to the CTAB method (Reichardt and Rogers 1997). DNA was dissolved and diluted in 0.1X TE (1 mM Tris, 0.1 mM EDTA, pH 8.0) to make a final concentration of 20 ng/μl for use as PCR templates.

Primer selection and polymerase chain reaction

A set of 40 pairs of SSR primers, evenly distributed among sorghum chromosomes (4 pairs for each chromosome from top to bottom, Table 3), was selected

Table 2. Morphological traits observed and countries collected for selected sorghum accessions

Accession	Country	Plant identification	AR	RR	ET	FT	GR	*R*
563053	Uganda	IS 8150	1.0	2.0	3	6	2	02
563151	Kenya	IS 8823	1.0	3.0	3	3	3	14
562982	Nigeria	EKA OR OKA BABA	1.0	2.0	3	6	2	02
563043	Japan	IS 8084	1.0	2.0	3	2	3	14
562960	Nigeria	KWASINI MUSA	1.0	2.0	4	3	3	03
562872	India	LAHAR KHURD	1.0	2.0	3	5	3	05
563431	United States	IS 10724	1.0	4.0	3	2	3	01
563249	South Africa	IS 9251	1.0	2.0	3	2	3	03
563164	Kenya	IS 8946	1.0	2.0	3	5	3	03
562824	India	SATPANI SHIDPUR	1.0	3.0	3	5	3	05
562970	Nigeria	FARAFARA	1.0	3.0	2	6	3	02
563131	Nigeria	IS 8714	1.0	1.0	3	2	3	03
562065	Burundi	GMN 10	1.0	3.5	3	6	3	11
267322	India	Red Hegari DL/59/1541	3.0	4.0	3	3	2	03
560388	South Africa	SA 1822	1.0	3.0	3	5	2	04
562818	India	MOVADA SAMADIYA	1.0	3.0	2	3	4	05
562253	Sudan	FAO 55035	1.0	4.0	3	5	2	03
562990	Nigeria	MURBAN GANGAU	1.0	2.0	3	6	3	02
562703	Mexico	FETERITA	1.0	2.0	3	2	4	03
562834	India	NATURA MAHAGOOTI	1.0	4.0	3	5	3	05
563399	United States	IS 10477	1.0	5.0	3	2	3	15
563463	United States	IS 10980	1.0	5.0	3	5	3	09
562724	United States	IS 806	1.0	2.0	3	2	4	11
514367	Senegal	Fela	1.0	4.5	4	6	5	05
586075	South Africa	RAMAHOTHLA	1.0	5.0	4	2	3	03
266952	China	1022	3.0	2.0	4	3	1	14
562252	Sudan	FAO 55034	1.0	4.0	5	3	4	03
563032	Japan	COLLIER 706C	1.0	3.0	2	5	3	01
564163	United States	na	na	na	na	na	na	na
585813	Mali	SEGUETENE	1.0	4.0	3	6	2	02
287595	Zimbabwe	Bahana 2/6	2.0	3.0	4	4	2	03
563269	South Africa	IS 9373	1.0	1.0	3	2	3	04
585312	India	IS 24400	1.0	3.5	3	5	3	12
563406	United States	TX 2525	1.0	5.0	3	2	3	15
563210	South Africa	IS 9179	1.0	1.0	5	3	3	03
563254	Uganda	COMBINE TYPE 3	1.0	1.0	4	5	3	03
585644	Mali	IS 25645	1.0	3.5	3	6	3	03
563549	China	Bin County Red Shell Baye	1.0	1.5	3	2	3	01
585341	South Africa	IS 24460	1.0	3.0	3	2	3	04
562739	India	IS 1035	1.0	2.0	3	5	4	05
585855	Mali	SAMBALA	1.0	3.0	3	4	3	05
586447	Hungary	DD WHITE SOONER	1.0	4.5	3	2	3	05
685739	Mali	KENINKE	1.0	4.0	3	5	2	02
585650	Mali	KENDE BABOU KOUKA	1.0	4.0	2	6	2	02
585795	Mali	IS 26007	1.0	2.0	2	6	1	02
236286	Australia	X8-6-2-3-8-2	1.0	4.0	3	3	2	04

(*Continued on next page*)

Table 2. (*continued*)

Accession	Country	Plant identification	AR	RR	ET	FT	GR	*R*
161586	Liberia	MN 2553	1.0	2.0	1	6	2	02
168501	Nigeria	Twagutage	1.0	2.5	4	4	2	03
BTX623	United States	na	na	na	na	na	na	na
ATX3042	United States	na	na	na	na	na	na	na
ATX623	United States	na	na	na	na	na	na	na
585787	Mali	TOUSSOU GARIMI	1.0	4.0	3	4	3	05
586482	South Africa	IS 27880	1.0	4.5	3	6	3	07
195039	Ethiopia	MN 3000	1.0	3.5	4	6	2	09
170794	Turkey	MN 2833	1.5	5.0	3	4	2	01
291377	China	IS 13567	1.0	3.5	2	2	2	06
291237	Jamaica	Vellai Cholam	1.5	4.0	3	6	4	05
291236	Jamaica	Ennai Cholam	1.5	3.5	3	4	3	05
253640	United States	TEXAS 660 (P.P.-CAPROCK)	1.0	3.5	3	3	2	04
248336	India	IS 1140	2.0	3.0	3	6	1	14
229861	South Africa	LADY FRERE RED	1.0	5.0	3	3	3	04
197542	Algeria	SUCRE DROME	1.0	4.5	3	2	2	01
221728	South Africa	SWAZI DWARF	1.0	5.0	3	3	2	03
229850	South Africa	IS 13056	1.5	5.0	3	4	2	04
253632	India	GUND. 34 RABI S.A. 6468	2.0	4.0	3	4	1	05
248334	India	IS 1138	2.0	3.0	3	6	1	14
267410	India	BELKO (S-76)	1.0	1.0	4	4	2	05
266955	South Korea	1257	2.0	4.0	5	3	1	13
267106	Former Soviet Union	K-64/II	3.0	4.0	4	4	3	05
267461	India	Wad Bejhir 51/56	1.0	2.0	4	5	4	03
267442	India	IS 2763	2.0	4.0	4	4	2	03
267593	Ethiopia	Zengada	3.0	5.0	5	6	1	03
267519	India	Framiola DL/59/1539	2.0	2.0	5	4	1	13
267515	India	Norghum USA DL/59/1535	2.0	3.0	4	1	1	03
267447	India	IS 2768	4.0	5.0	1	6	1	02
291382	China	282	1.0	2.5	3	1	2	17
297129	Uganda	IS 13612	1.0	2.5	3	6	2	03
453035	Ethiopia	ETS 2273	1.0	4.5	3	6	5	05
297094	Uganda	E-2	1.0	1.5	5	6	1	03
453831	Ethiopia	ETS 2678	1.0	3.5	3	6	4	05
453691	Ethiopia	ETS 2611	2.0	4.0	3	6	5	05
513996	Benin	Gaouri Daneri	3.5	4.0	2	6	4	02
455013	Ethiopia	ETS 3349	1.0	2.0	3	6	4	05
514120	Benin	Tokobessenou	3.5	1.5	2	6	3	02
514015	Benin	Esse Tinha	2.5	3.5	3	6	3	02
267633	Ethiopia	IS 3046	4.0	3.0	4	6	1	09
282829	Chad	Nadjada	4.0	4.0	4	4	2	03
287598	Zimbabwe	Bahana 25/7	2.0	3.0	4	4	2	03
284971	Argentina	Adjigoni	2.0	2.0	4	4	2	05
267643	Ethiopia	Cullubi	2.0	3.0	3	6	1	13
287597	Zimbabwe	Bahana 2/8	3.0	2.0	4	4	2	13
287578	Zimbabwe	Feterita Wad Umm Benein	3.0	4.0	4	4	2	14
285194	Nigeria	Nunaba	4.0	1.0	3	6	1	01
266063	Guatemala	6915	5.0	3.0	3	6	1	05
514140	Benin	Sg. 4285	1.0	1.5	4	6	4	12
521702	Malawi	Kashonthe	1.0	2.5	2	4	1	02

AR: anthracnose response; RR: rust response; ET: endosperm texture; FT: flowering time; GR: grain mold response; R: race. na: no data available for these accessions.

Table 3. Information for the selected sorghum SSR markers from website

SSR marker	LG	cM	Repeat	No. of alleles	Temp (°C)	Size of amplicons (bp)
Xtxp 325*	A_T1	27.3	(AAT)23	5	55	247
Xtxp 88	A_T2	110	(AG)31	6	53	144
Xtxp 279	A_B1	178.6	(CTT)19	4	55	276
Xtxp 46	A_B2	242.9	(GT)10	3	55	253
Xtxp 63	B_T1	8.3	(GA)24	5	55	204
Xtxp 201	B_T2	76.6	(GA)36	6	66	222
Xtxp 100*	B_B1	162.7	(CT)19	2	55	117
Xtxp 8	B_B2	201.4	(TG)31	6	60	148
Xtxp 266	C_T1	11.9	(GT)8	2	55	197
Xtxp 33	C_T2	80	(TC)20+(TG)5+(CT)9+(TG)7	8	55	221
Xtxp 114	C_B1	135	(AGG)8	2	50	234
Xtxp 69	C_B2	182.3	(CT)12	4	50	188
Xtxp 12	D_T1	100	(CT)22	4	55	193
Xtxp 41	D_T2	131.2	(CT)19	5	55	278
Xtxp 212*	D_B1	145	(GT)10	3	55	150
Xtxp 21	D_B2	171.1	(AG)18	5	60	179
Xtxp 40	E_T1	0	(GGA)7	2	55	138
Xtxp 312	E_T2	48.2	(CAA)26	9	55	192
Xcup 47	E_B1	?	(GA)21	6	50	164
Xtxp 168	E_B2	159.6	(AC)10	3	55	178
Xtxp 339	F_T1	37.5	(GGA)7	2	55	202
Xtxp 10	F_T2	69.5	(CT)10	5	50	145
Xtxp 287	F_B1	113.7	(AAC)21	5	55	367
Xtxp 289	F_B2	157.3	(CTT)16+(AGG)6	5	55	290
Xtxp 20	G_T1	72	(AG)21	5	60	217
Xtxp 270	G_T2	73	(GAA)33+(GAAA)6+(GTA)11	6	55	279
Xtxp 331	G_B1	75.9	GAT(32)	4	55	226
Xtxp 141*	G_B2	136	(GA)23	5	55	163
Xtxp 273	H_T1	0	(TTG)20	5	55	223
Xtxp 210	H_T2	30	(CT)10	6	55	188
Xtxp 354	H_B1	99.2	(GA)21+(AAG)3	4	55	157
Xtxp 105	H_B2	126.1	(TG)5+(CT)6+(GT)7	3	55	291
Xtxp 6	I_T1	0	(CT)33	9	50	120
Xtxp 317	I_T2	61.1	(CCT)5+(CAT)11	3	55	162
Xtxp 176	I_B1	87.8	(AG)4+(GA)4	2	55	161
Xtxp 17	I_B2	105.6	(TC)16+(AG)12	3	55	164
Xtxp 65	J_T1	7.4	(ACC)4+(CCA)3+(CT)8	4	55	128
Xtxp 303	J_T2	33.1	(GT)13	4	55	160
Xtxp 225	J_B1	53.8	(CT)9+(CA)14	4	55	165
Xtxp 262	J_B2	115	(GT)5	2	55	167

*SSR marker derived from EST-SSRs. LG: Linkage group from A to J; T stands for top and B for bottom.
Temp: annealing temperatur for PCR. ?: unknown mapping location in cM.

with known amplicon sizes of 117–367 bp from the sorghum database (http://sorghumgenome.tamu.edu, Bhattramaki et al., 2000) and new additional SSR markers converted from RFLPs (Schloss et al., 2002). All the primers (the forward one fluorescently labeled either blue, or green or black for the *Beckman* CEQ8000 analysis) were obtained from *Proligo* LLC (Boulder, CO, USA, http://www.proligo.com).

The PCR was performed in a PCR iCycler (*Bio-RAD*) with a 96-well plate format. Ten ng of genomic DNA were used in a 12.5 μl total volume with a final concentration of 1X PCR buffer, 0.8 pmol/μl each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 0.05 U/μl *Taq* DNA polymerase (*Promega*). The final concentration of the forward primers in the reaction was adjusted based on the fluorescent dye emission intensity in order to obtain proper height of peaks on the *Beckman* CEQ8000 chromatogram. The PCR was programmed as denaturing at 96°C for 1 min; 30 cycles (96°C for 10 sec, 50∼60°C for 20 sec, 72°C for 30 sec); extending at 72°C for 2 min and storing at 4°C. Variable PCR annealing temperatures (Table 3) were used depending on the calculated Tm (melting temperature) of primers. If the PCR products could not be separated on Beckman CEQ8000 on the same day, they were stored at 4°C for use within two days or −20°C for use within two weeks. To ensure that the results were reproducible, each of the amplifications was repeated twice.

Capillary electrophoresis and fragment analysis

For the capillary electrophoresis, one μl of PCR products from each of three pairs of SSR primers (in a combination of blue, green and black) were transferred into a Beckman 96-well plate by multiple channel pipette, mixed with 20 μl of sample loading solution, 0.25 μl of size standard 400, and overlaid with one drop of mineral oil. In terms of efficiency (reducing cost and increasing throughput), amplified DNA fragments by three pairs of primers from one accession were separated in one capillary on Beckman CEQ8000 and shown on the same chromatogram (Figure 1). Each peak was produced by the accumulation of dye signal from the same kind of labeled amplicons (or fragments). The height of a peak reflects the number of corresponding fragments at the position. Clear peaks on the chromatograms were interpreted into sized fragments referencing the size standard 400 by the Beckman software. Polymorphic peaks from different accessions were used for genetic data analysis.

Data analysis

The data from polymorphic traits of six morphological markers on GRIN (Table 2) and data from polymorphic peaks of 40 SSR markers on CEQ8000 were used for genetic analysis, respectively. Since fragment amplification from microsatellite sequence by *Taq* DNA polymerase can lead to slippage and cause insertion-deletion mutations (Shinde et al., 2003), prior to the genetic analysis some sized fragments analyzed by Beckman software were edited manually. The edited data were then analyzed using a computer program, Microsat (version 1.5b) for calculating a distance matrix (proportion of shared alleles) on the microsatellite allele data using the actual allele sizes (Minch et al., 1995, 1996). A phylogenetic tree was constructed from the distance matrix and a coefficient of similarity was calculated using the phylogeny inference package (PHYLIP) software (options, NJ) (Felsenstein, 2004). The phylogenetic tree was visualized using TREEVIEW software (Page, 1996). A dendrogram from the morphological data was generated using shared characters with all the characters being given equal weight.

Polymorphism information content (PIC) provides an estimate of the discriminatory power of a locus or loci by the number of alleles expressed and the relative frequencies of those alleles. PIC values from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency) were calculated by adapting the following method (Botstein et al., 1980; Anderson et al., 1993).

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where P_{ij} is the frequency of the jth allele for the SSR marker i, and the summation extends over n alleles.

Results and discussion

SSR profile

As an example, the peaks from three SSR markers: Xtxp141, Xtxp266 and Xtxp273 were shown in order from left to right on the same chromatograms, and the peaks from four different accessions: PI 563131, 562065, 267519 and 267515 were shown on different chromatograms in Figure 1A, 1B, 1C and 1D, respectively. The peaks from the marker Xtxp266 (the second peaks) and Xtxp273 (the third peaks) were

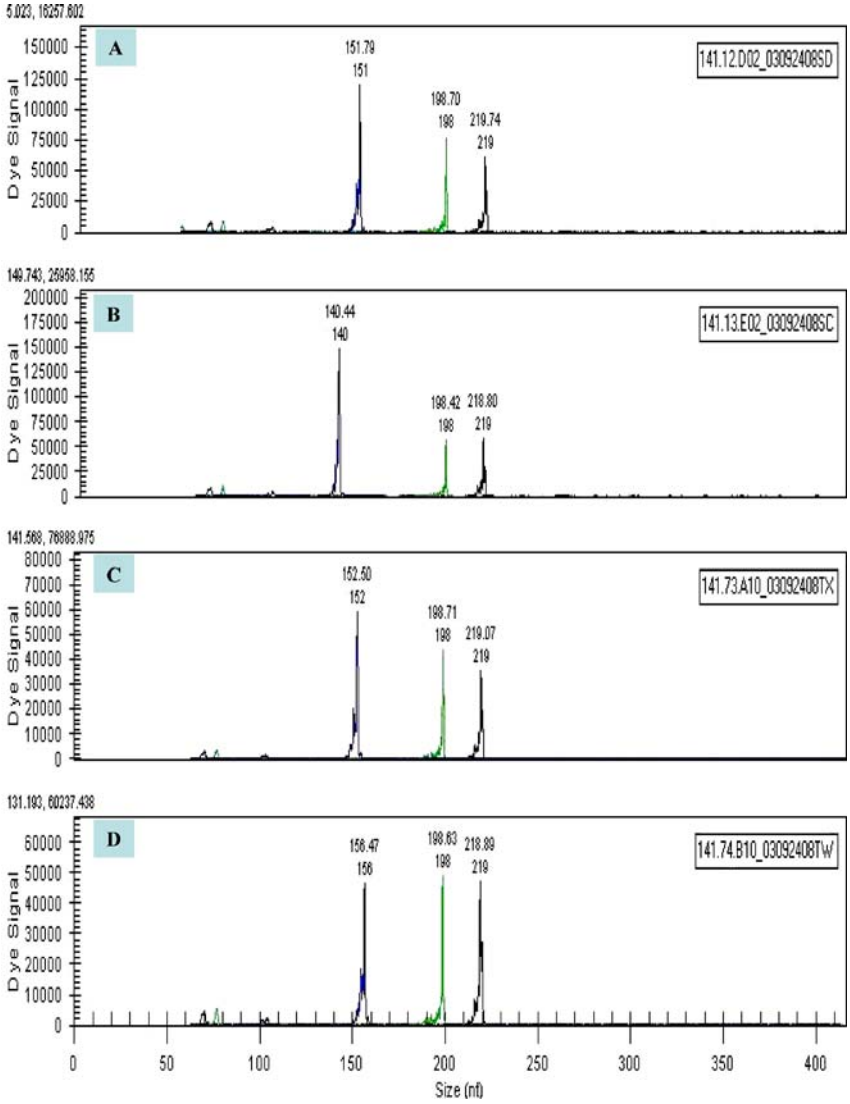


Figure 1. Stacking chromatograms show examples of polymorphic and non-polymorphic amplicons separated by the capillary electrophoresis (Beckman CEQ8000). Panel **A**, **B**, **C**, and **D** is the chromatogram showing separated amplicons amplified by three markers (**Xtxp141**, **Xtxp266** and **Xtxp273**) from accession PI 563131, 562065, 267519 and 267515, respectively. X axis is the size of nucleotides; Y axis is the dye signal intensity and each sharp peak represents a fragment with the corresponding size (bp) on the top of the peak.

monomorphic whereas the first peaks from the marker Xtxp141 were polymorphic for these four accessions. The size of polymorphic fragments was 152, 140, 152 and 156 base pairs (bp), respectively. Due to the nature of *Taq* DNA polymerase slippage on the SSR sequence (Shinde et al., 2003), one base pair could be off from the real fragment length call. For example, although the length of fragments (stutter products) amplified by Xtxp141 from accessions PI 563131 and 267519 was called at 151.79 bp (the first peak in Figure 1A) and 152.50 bp (the first peak in Figure 1C) respectively, they were scored into the same allele (152 bp) after editing. This demonstrates how raw data were edited before the alleles were used for the genetic data analysis. In total, 414 polymorphic peaks were identified. The average number of alleles per marker were 10.35 (ranging from 2 to 33 alleles). The distinguishing power obtained from these accessions for each SSR marker varied greatly. The mean PIC value was 0.8228 (ranging from 0.0887 to 0.9461).

Characterization by molecular genetic data

A dendrogram from the genetic data was generated using accession PI 563043 from Japan as an outgroup (Figure 2). To check internal controls (i.e. two lines were the same but requested from different sources), accession PI 564163 and BTX623 were clustered at the same position (99% similarity), and ATX623 (a male sterile line of BTX623) was almost genetically identical to these two accessions and clustering into the same group. This confirmed the reliability of our genetic data and the genetic classification from the data should be accurate. To observe whether the genetic classification relates to disease responses and geographical origin (or collection site) of accessions, information on the disease response and the geographically collected location of accessions were listed in parallel on Figure 2. The genetic dendrogram demonstrates that the accessions can be classified into three main clades (I, II and III) and nine subgroups (from 1 to 9).

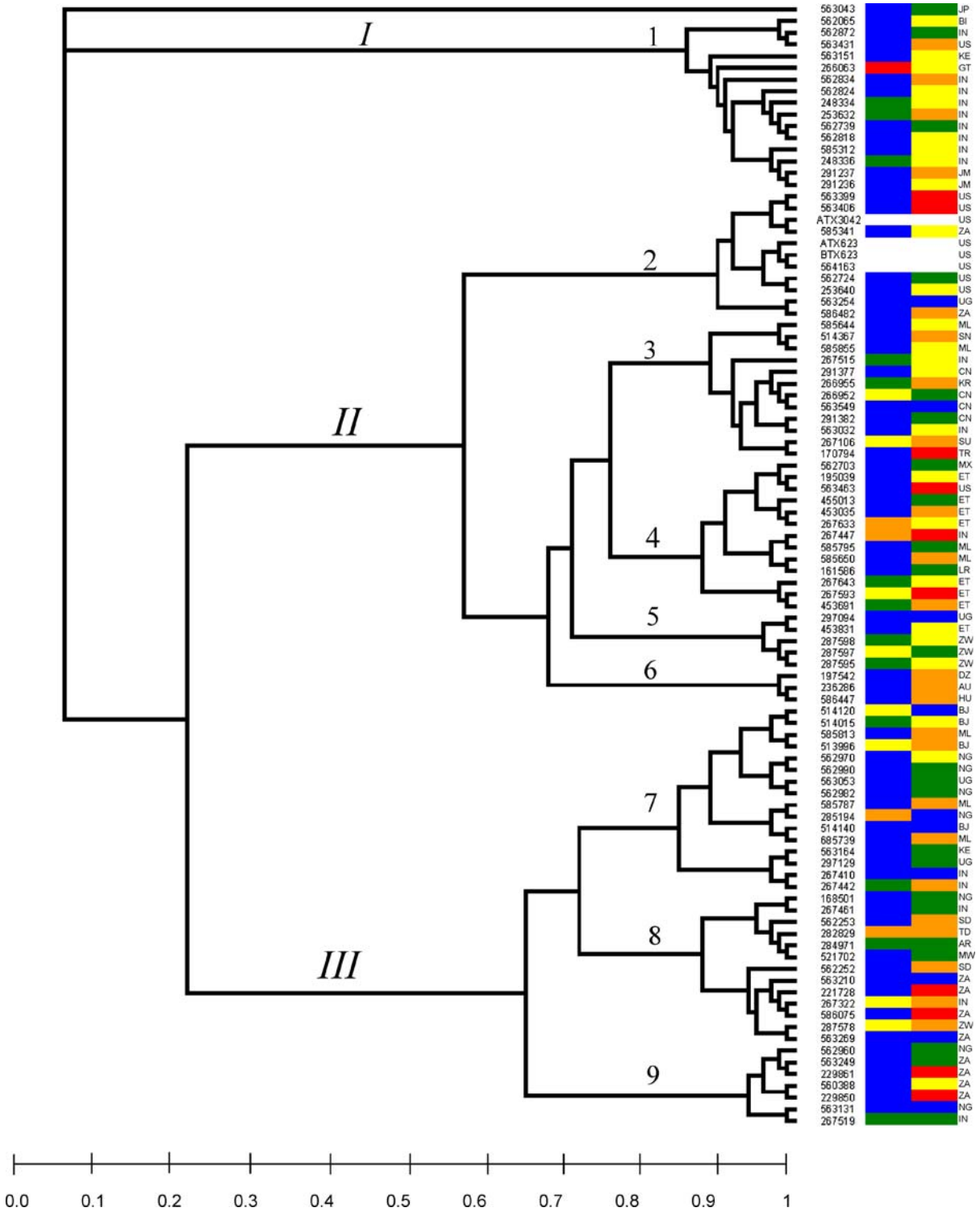
Clade I contained one subgroup consisting of 15 accessions. Two accessions (PI 291236 and 291237) from Jamaica were nearly identical genetically and had similar responses to anthracnose (highly resistance) but different responses to rust. Nine out of 15 accessions were collected from India and they were all resistance to anthracnose however, they showed different responses to rust (from resistant to susceptible). Subgroup 1 can be mainly classified as India group but there were also accessions from other countries (BI, US, KE, GT and JM). Within Clade I, no accessions were highly resistant to rust. From the sorghum race classification, most of the accessions (9 out of 15) belonged to Durra (race code 05 = Durra, Table 2).

Clade II contained five subgroups consisting of 44 accessions. Subgroup 2 contained 11 accessions mainly representing collections from the US (8 out of 11). All tested accessions from the US were highly resistance to anthracnose but responded to rust from resistant to highly susceptible. Interestingly, two male sterile lines (ATX3042 and ATX623) were classified into the same subgroup suggesting little genetic difference exists between these two male sterile lines. Subgroup 3 contained 12 accessions. All accessions from China clustered into this subgroup and they were resistant to either anthracnose or rust and/or to both fungal diseases. This is consistent with previous studies that Chinese sorghums may have a relatively narrow genetic base (Oliveira et al., 1996, Yang et al., 1996). Subgroup 4 contained 13 accessions, 10 of which were from the Africa collection (Ethiopia, Mali and Liberia). There was a broad spectrum of responses to anthracnose and rust in this subgroup. Subgroup 5 contained five accessions and all of them were from Africa. There were no accessions in this subgroup highly susceptible to either anthracnose or rust. Subgroup 6 contained three accessions all of which were highly resistant to anthracnose but susceptible to rust.

Clade III contained three subgroups consisting of 36 accessions. Subgroup 7 contained 16 accessions, 14 of which were collected from Africa. Within this subgroup, eight accessions (from PI 514120 to 562982) were closely clustered together and classified as the same race, Guinea (race code 02, Table 2) but they had different responses to anthracnose and rust. Subgroup 8 contained 13 accessions (mainly from Africa and South Africa) 9 of which belonged to race Caudatum (race code 03) and there was a big variation among these accessions to rust response (from highly resistant to highly susceptible). Subgroup 9 contained seven accessions mainly representing accessions from South Africa and they were highly resistant to anthracnose but with variable responses to rust.

Overall, the accessions from South Africa and Mali were highly resistant to anthracnose. Eight out of the ten accessions highly resistant to rust were from either South Africa or Africa. The branched subgroups in general, correlated very well with the locations where the accessions were collected. Our results reconfirmed that geographical origin of accessions was associated with the genetic similarity of sorghums (Morden et al., 1989; Tao et al., 1993; Deu et al., 1994). However, there was

Figure 2. A dendrogram generated from SSR genotyping for clustering 96 sorghum germplasm accessions using the similarity coefficient and the neighbor joining clustering method. On the right of the dendrogram, the first column is the accession number, the second column is the color bar for anthracnose response, the third column is the color bar for rust response and the fourth column is the abbreviation for the countries of origin or collection. The color bars for disease resistance are blue representing for highly resistant; green for resistant, yellow for intermediate, orange for susceptible and red for highly susceptible. The abbreviations for the collected countries are as follows: DZ, Algeria; AR, Argentina; AU, Australia; BJ, Benin; BI, Burundi; CN, China; ET, Ethiopia; GT, Guatemala; HU, Hungary; IN, India; JP, Japan; JM, Jamaica; KE, Kenya; KR, South Korea; LR, Liberia; ML, Mali; MX, Mexico; MW, Malawi; NG, Nigeria; SD, Sudan; SN, Senegal; SU, Former Soviet Union; TD, Chad; TR, Turkey; UG, Uganda; US, United States; ZA, South Africa; ZW, Zimbabwe. The coefficient of similarity has been used below the 0 to 1 scale in the figure.


I
II
III
1
2
3
4
5
6
7
8
9
563043 JP
562065 BI
562872 IN
563431 US
563151 KE
266063 GT
562834 IN
562824 IN
248334 IN
253632 IN
562739 IN
562818 IN
585312 IN
248336 IN
291237 JM
291236 JM
563399 US
563406 US
ATX3042 US
585341 ZA
ATX623 US
BTX623 US
564163 US
562724 US
253640 US
563254 UG
586482 ZA
585644 ML
514367 SN
585855 ML
267515 IN
291377 CN
266955 KR
266952 CN
563549 CN
291382 CN
563032 IN
267106 SU
170794 TR
562703 MX
195039 ET
563463 US
455013 ET
453035 ET
267633 ET
267447 IN
585795 ML
585650 ML
161586 LR
267643 ET
267593 ET
453691 ET
297094 UG
453831 ET
287598 ZW
287597 ZW
287595 ZW
197542 DZ
236286 AU
586447 HU
514120 BJ
514015 BJ
585813 ML
513996 BJ
562970 NG
562990 NG
563053 UG
562982 NG
585787 ML
285194 NG
514140 BJ
685739 ML
563164 KE
297129 UG
267410 IN
267442 IN
168501 NG
267461 IN
562253 SD
282829 TD
284971 AR
521702 MW
562252 SD
563210 ZA
221728 ZA
267322 IN
586075 ZA
287578 ZW
563269 ZA
562960 NG
563249 ZA
229861 ZA
560388 ZA
229850 ZA
563131 NG
267519 IN
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

no very clear correlation between the genetic classification and the observed responses of these accessions to anthracnose and rust.

Characterization by morphological data

Six morphological traits were used for characterization of the 92 accessions. Although some morphological traits are quantitative (e.g. flowering time), all of them were scored qualitatively. The scope of scoring scale was from 5 to 14 (with average 7.83 scales per trait). A dendrogram was generated from the morphological data (Figure not shown). The accessions were classified into 12 groups. Accessions within some groups were all resistant to anthracnose. Accessions from one group all belonged to Durra (race code 05). Overall, there was no clear correlation between classified groups and accession-collected locations from the morphological dendrogram. Since there are over 40,000 accessions in the sorghum collection, it was difficult to obtain morphological data for all accessions from the same environment (year and location). Some morphological data were collected from the same locations but from different years. Morphological traits (for example, flowering time) were greatly affected by the environment. It was found from another study (Dahlberg et al., 2002) that the correlations between agronomic descriptors and RAPD data were not high. Ideally, it would be informative to observe these six morphological traits for all 96 accessions in a well-designed experiment (for example, two locations $\times$ two years). However, in reality, it is very challenging to observe all six traits for all 96 accessions because some photoperiod-sensitive accessions from certain locations did not flower. Additionally, due to inoculation, one well-developed disease could overwhelm and shadow the development of another disease. Therefore, to observe and evaluate anthracnose and rust disease responses at the same location and year needs a specific experimental design. Because some of our morphological data were collected at different locations and years, the morphological and genetic data were not integrated for further analysis.

Utilization for germplasm conservation and cultivar improvement

One of the big challenges for germplasm management is to identify redundant accessions. Minimizing the number of germplasm accessions can reduce the cost for regeneration and maintenance (Dean et al., 1999) as well as help focus on non-redundant accessions for research and utilization. In this pilot experiment, 96 accessions have been genetically classified into three clades and nine subgroups. Furthermore, there seems not much genetic divergence (less than 1–2%) for 35 pairs of accessions (genetic similarity over 99%, accessions with the shortest internodes which are similar to accessions BTX623 and 564163 in Figure 2). This information could be useful for germplasm conservation and cultivar improvement. However, some of the pairs (for example, PI 266952 and PI 563594; PI 267106 and PI 170794) showed different responses to both anthracnose and rust. When minimizing the number of germplasm accessions by the use of limited molecular marker data, caution has to be taken and morphological data (here it is response to fungi) have to be taken into account.

Sorghum improvement programs largely depend on genetic resources and their characterization and evaluation information. Two male sterile lines (ATX623 and ATX3042) from the US were characterized and were very divergent from accessions from South Africa in Clade III. To a certain extent, hybrid heterosis depends on the genetic distance between parents. In order to obtain maximum heterosis, the probability of heterosis would be high for making hybrids between parents from US sorghum and from South Africa sorghum (in Clade III). Resistance to rust and anthracnose was genetically classified into two and three clades, respectively. In theory, each clade could contain different resistance sources (alleles of resistance genes and/or the resistance genes). The genes and/or alleles of genes contributing to the resistance from each clade or subgroup could be diversified and considered as different resistant sources. For the selection of parents to make a cross for mapping genes for anthracnose resistance, genetically divergent accessions should be used except for the difference in response to anthracnose. For example, US sorghum BTX623 is highly susceptible (Boora et al., 1998) and South Africa sorghum PI 229850 (Figure 2) is highly resistant to anthracnose. In addition, they are very divergent genetically (the common bands shared by these two genotypes were only 22%; the pairwise distance of these two genotypes was 0.78). It would be ideal to use these two accessions as mapping parents to make a cross for generation of a segregation population. Since they are very divergent, it is possible that many markers can be mapped onto the sorghum genome by using the segregation population to define the corresponding chromosome regions where the resistance genes are located. For cultivar improvement, sorghum breeders can introduce resistant

sources from both the India and South Africa subgroups by crossing to enhance the resistance to anthracnose. Genes for rust and anthracnose resistance have not been identified and cloned in sorghum. However, genes for rust resistance have been cloned in other cereal crops (Collins et al., 1998, 1999; Brueggeman et al., 2002; Feuillet et al., 2003; Huang et al., 2003). Orthologs and paralogs for rust resistance might be identified from sorghum germplasm. To associate the orthologs or paralogs with the phenotypes for the rust response might help to identify genes and genetic diversity for sorghum rust resistance.

Disclaimer

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Acknowledgments

The authors gratefully thank Drs. James W. Buck and C.J. Chang for critical reading of the manuscript, three anonymous reviewers for useful comments on the manuscript and Ms. Meredith Reed for her excellent assistance for the Beckman analysis, Ms. Merrelyn Spinks for searching the GRIN database, Ms. Lee Ann Chalkley, Ms. Phiffie Vankus and Mr. David Pinnow for providing and germinating sorghum seeds.

References

Anderson, J.A., G.A. Churchill, J.E. Autrique, S.D. Tanksley & M.E. Sorrells, 1993. Optimizing parental selection for genetic linkage maps. Genome 36: 181–186.

Bhattramakki, D., J. Dong, A.K. Chhabra & G.E. Hart, 2000. An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43: 988–1002.

Boora, K.S., R. Frederiksen & C. Magill, 1998. DNA-based markers for a recessive gene conferring anthracnose resistance in sorghum. Crop Sci. 38: 1708–1709.

Botstein, D., R. White, M. Skolnick & R. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314–331.

Brueggeman, R., N. Rostoks, D. Kudrna, A. Kilian, F. Han, J. Chen, A. Druka & B. Steffenson, 2002. The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99: 9328–9333.

Casela, C.R., R.A. Frederiksen & A.S. Fereira, 1993. Evidence for dilatory resistance to anthracnose in sorghum. Plant Dis 77: 908–911.

Collins, N., J. Drake, M. Ayliffe, Q. Sun, J. Ellis, S. Hulbert & T. Pryor, 1999. Molecular characterization of the maize *Rp1-D* rust resistance haplotype and its mutants. Plant Cell 11: 1365–1376.

Collins, N.C., C.A. Webb, S. Seah, J.G. Ellis, S.H. Hulbert & A. Pryor, 1998. The isolation and mapping of disease resistance gene analogs in maize. Mol Plant-Microbe Interact 11: 968–978.

Dahlberg, J.A., X. Zhang, G.E. Hart & J.E. Mullet, 2002. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurements. Crop Sci 42: 291–296.

Dean, R.E., J.A. Dahlberg, M.S. Hopkins, S.E. Mitchell & S.Kresovich, 1999. Genetic redundancy and diversity among 'orange' accessions in the U.S. national sorghum collection as assessed with simple sequence repeat (SSR) markers. Crop Sci 39: 1215–1221.

Deu, M., D. Gonzales-de-Leon, J.-C. Glaszmann, I. Degremont, J. Chantereau, C. Lanaud & P. Hanon, 1994. RFLP diversity in cultivated sorghum in relation to racial differentiation. Theor Appl Genet 88: 834–844.

Feuillet, C., S. Travella, N. Stein, L. Albar, A. Nublat & B. Keller, 2003. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100: 15253–15258.

Felsenstein, J., 2004. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Frederiksen, R.A., 2000. Diseases and disease management in sorghum. In: C.W. Smith & R.A. Frederiksen (Eds.), Sorghum origin, history, technology, and production, pp. 497–533. John Wiley & Sons, Inc., New York.

Huang, L., S.A. Brooks, W. Li, J.P. Fellers, H.N. Trick and B.S. Gill, 2003. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploidy genome of bread wheat. Genetics 164: 655–664.

Minch, E., A. Ruiz-Linares, D. Goldstein, M. Feldman & L.L. Cavalli-Sforza, 1995, 1996. Microsat (version 1.5b): A computer program for calculation various statistics on microsatellite allele data. WWW: http://lotka.stanford.edu/microsat.html.

Morden, C.W., J. Doebley & Schertz K. F. 1989. Allozyme variation in old world races of *Sorghum bicolor* (Poaceae). Am J Bot 76: 247–255.

Oliveira, A.C., T. Richter & J.L. Bennetzen, 1996. Regional and racial specificities in sorghum germplasm assessed with DNA markers. Genome 39: 579–587.

Page, R.D.M., 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in Biosciences 12: 357–358.

Panday, S., A. Sindhu & K.S. Boora, 2002. RAPD based DNA markers linked to anthracnose disease resistance in *Sorghum bicolor* (L.) Moench. Indian J Exp Biol 40: 206–211.

Rana, B.S., D.P. Tripathi & N.G. Rao, 1976. Genetic analysis of some exotic x Indian crosses in sorghum. XV. Inheritance of resistance to sorghum rust. Indian J Genet Plant Breed 36: 244–249.

Reichardt, M. & S. Rogers, 1997. Preparation of genomic DNA from plant tissue. In: F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith & K. Struhl (Eds.), Current

protocols in molecular biology, pp 2.3.3–7. John Wiley & Sons, Inc., New York.

Schlötterer, C., 2004. The evolution of molecular markers – just a matter of fashion? Nature Rev Genet 5: 63–69.

Schloss, S.J., S.E. Mitchell, G.M. White, R. Kukatla, J.E. Browers, A.H. Paterson & S. Kresovich, 2002. Characterization of RFLP probe sequences for genetic discovery and SSR development in *Sorghum bicolor* (L.) moench. Theor Appl Genet 105: 912–920.

Shinde, D., Y. Lai, F. Sun & N. Arnheim, 2003. *Taq* DNA polymerase slippage mutation rates measured by PCR and quasi-likelihood analysis: (CA/GT)n and (A/T)n microsatellites. Nucleic Acids Res 31: 974–980.

Thomas, M.D., I. Sissoko & M. Sacko, 1996. Development of leaf anthracnose and its effect on yield and grain weight of sorghum in West Africa. Plant Dis 79: 151–153.

Tao, Y.Z., D.R. Jordan, R.G. Henzell & C.L. McIntyre, 1998. Identification of genomic regions for rust resistance in sorghum. Euphytica 103: 287–292.

Tao, Y., J. M. Manners, M.M. Ludlow & R.G. Henzell, 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). Theor Appl Genet 86: 679–688.

Yang, W., A.C. de Oliveira, I. Godwin, K. Schertz & J.L. Bennetzen, 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: Variability in Chinese sorghums. Crop Sci 36: 1669–1676.